

IN THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

1. (Original) Method for the chromatographic separation of a nucleic acid mixture wherein plasmid DNA is separated from other components of the mixture, especially other nucleic acids, characterised in that

- b) as appropriate the nucleic acid mixture is adjusted with one or more alkali salts and/or alkaline earth salts in aqueous solution to a conductance that is equivalent to a conductance of 70 mS to 95 mS at a pH of 4.8 to 5.4 at a temperature of 20°C, and
- b) the nucleic acid mixture is brought into contact with a chromatographic stationary phase,
- c) the stationary phase is then washed at least once with a solution comprising an alkali salt in a concentration range of 900 mM to 1800 mM based on a pH of 7 to 7.4 and/or an alkaline earth salt in a concentration range of 100 mM to 240 mM based on a pH of 7 to 7.4, and
- d) the plasmid DNA bound to the chromatographic stationary phase is subsequently eluted with a solution comprising an alkali salt in a concentration of 1300 mM or higher based on a pH of 7 to 7.4 and/or an alkaline earth salt in a concentration of 270 mM or higher based on a pH of 7 to 7.4.

2. (Original) Method as described in claim 1, characterised in that the alkali salt is an alkali halide and the alkaline earth salt is an alkaline earth halide.

3. (Original) Method as described in claim 2, characterised in that the alkali halide is NaCl, KCl, CsCl and/or LiCl and the alkaline earth halide is CaCl₂.

4. (Currently Amended) Method as described in ~~claims 1 to 3~~ claim 1, characterised in that the nucleic acid mixture is adjusted with KCl to a conductance that is equivalent to a conductance of 70 mS to 85 mS at a pH of 4.8 to 5.4 and a temperature of 20°C.

5. (Original) Method as described in claim 4, characterised in that the nucleic acid mixture is adjusted with KCl to a conductance that corresponds to a conductance of 70 mS to 80 mS at a pH of 4.8 to 5.4 and a temperature of 20°C.

6. (Currently Amended) Method as described in ~~claims 1 to 3~~ claim 1, characterised in that the nucleic acid mixture is adjusted with NaCl to a conductance that corresponds to a conductance of 70 mS to 95 mS at a pH of 4.8 to 5.4 and a temperature of 20°C.

7. (Original) Method as described in claim 6, characterised in that the nucleic acid mixture is adjusted with NaCl to a conductance that corresponds to a conductance of 85 mS to 95 mS at a pH of 4.8 to 5.4 and a temperature of 20°C.

8. (Currently Amended) Method according to ~~claims 1 to 7~~ claim 1, characterised in that the washing step/s from step b) of claim 1 is/are carried out with a solution comprising KCl in a concentration range of 1100 mM to 1800 mM based on a pH of 7 to 7.4.

9. (Original) Method according to claim 8, characterised in that the washing step/s from step b) of claim 1 is/are carried out with a solution comprising KCl in a concentration range of 1300 mM to 1700 mM relating to a pH of 7 to 7.4.

10. (Currently Amended) Method according to ~~claims 1 to 7~~ claim 1, characterised in that the washing step/s from step b) of claim 1 is/are carried out with a solution comprising KCl in a concentration range of 950 mM to 1200 mM based on a pH of 7 to 7.4.

11. (Original) Method according to claim 10, characterised in that the washing step/s from step b) of claim 1 is/are carried out with a solution comprising NaCl in a concentration range of 1100 mM to 1150 mM based on a pH of 7 to 7.4.

12. (Currently Amended) Method as described in ~~claims 1 to 11~~ claim 1, characterised in that the elution step from step c) from claim 1 is carried out with a solution comprising KCl in a concentration of 1900 mM or higher based on a pH of 7 to 7.4.

13. (Currently Amended) Method as described in ~~claims 1 to 11~~ claim 1, characterised in that the elution step from step c) from claim 1 is carried out with a solution comprising NaCl in a concentration of 1300 mM or higher based on a pH of 7 to 7.4.

14. (Currently Amended) Method as described in ~~claims 1 to 13~~ claim 1, characterised in that the chromatographic stationary phase is an anion exchanger.

15. (Currently Amended) Method as described in ~~claims 1 to 14~~ claim 1, characterised in that silica gel, diatomaceous earth, glass, aluminium oxide, titanium oxide, zirconium oxide, hydroxy apatite, dextran, agarose, acrylamide, polystyrene resin or copolymers of the named materials are used as chromatographic stationary phase.

16. (Original) Method as described in claim 15, characterised in that the chromatographic stationary phase is obtainable by reaction of one of the stationary phase named in claim 15 in a first step with a silanisation reagent of the general structure I

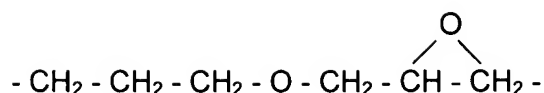


wherein

R^1 is an alkoxy residue with 1 to 10 C atoms, especially $-OCH_3$, $-OC_2H_5$ or $-OC_3H_7$, or a halogen atom, especially $-Cl$, or a dialkylamino group with identical or different alkyl residues with 1 to 6 C atoms;

R^2 and R^3 independently of one another are hydrocarbon residues with 1 to 10 C atoms, especially $-CH_3$, $-C_2H_5$ or $-C_3H_7$, or an alkoxy residue with 1 to 10 C atoms, especially $-OCH_3$, $-OC_2H_5$ or $-OC_3H_7$, or a halogen atom or an alkyl residue with 4 to 20 carbon atoms interrupted by at least one oxygen atom or amino groups, wherein this residue can also be substituted once or several times by halogen, cyano, nitro, amino, monoalkylamino, dialkylamino, hydroxy or aryl;

R^4 is a hydrocarbon chain with 1 to 20 C atoms or an alkyl residue interrupted by at least one oxygen atom or amino group, whereby this residue can also be substituted one or several times with halogen, cyano, nitro, amino, monoalkylamino, dialkylamino, alkoxy, hydroxy, aryl and/or epoxy, especially



followed by a second step wherein the stationary phase modified in the first step is reacted with a reagent of the general structure II



wherein

X is an amino-, hydroxy-, epoxy group or a halogen atom,

R is a hydrocarbon chain with 2 to 20 C atoms or an alkyl residue interrupted by at least one oxygen atom or amino group, where in this residue can also be substituted once or several times by halogen, cyano, nitro, amino, monoalkylamino, dialkylamino, alkoxy, hydroxy, aryl and/or epoxy,

Y is a hydrocarbon residue with anion exchange forming functional groups with 1 to 10 C atoms that can be substituted once or several times by amino-, monoalkylamino-, dialkylamino-, trialkylammonium.

17. (Currently Amended) Method as described in ~~claims 1 to 16~~ claim 1, characterised in that the method is carried out at room temperature.

18. (Currently Amended) Method as described in ~~claims 1 to 3~~ claim 1, characterised in that at least in step b) of claim 1 KCl is used as salt.

19. (Original) Method as described in claim 1, characterised in that mixtures of different alkali salts and/or alkaline earth salts can also be used in the steps a), c) and d) of claim 1.

20. (Currently Amended) Method as described in ~~claims 1 to 19~~ claim 1, characterised in that the nucleic acid mixture is a cleared lysate from prokaryotic cells.

21. (Currently Amended) Use of the method as described in ~~claims 1 to 20~~ claim 1, for the purification of plasmid DNA.

22. (Currently Amended) Use of plasmids obtained by means of a method as described in ~~claims 1 to 20~~ claim 1 for the preparation of an agent containing plasmid DNA for use in gene therapy or genetic vaccination.